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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

Dean et al.

Application No. 09/514,113

Confirmation No. 9257

Filed: February 28, 2000

For: "METHOD FOR REDUCING ARTIFACTS IN NUCLEIC ACID AMPLIFICATION"

Examiner: Sisson, Bradley L.

Art Unit: 1655

AMENDMENT AND RESPONSE TO OFFICE ACTION

Commissioner for Patents
Washington, D.C. 20231

NEEDLE & ROSENBERG, P.C.
The Candler Building
127 Peachtree Street, N.E.
Atlanta, Georgia 30303-1811

Sir:

Responsive to the Office Action mailed on November 27, 2001, please amend the application as follows.

Amendment

In the Claims

Please rewrite the following claims.

B1

1. (Amended) A method of reducing formation of artifacts in a nucleic acid amplification reaction, the method comprising

using a template-deficient oligonucleotide as [at least one of the oligonucleotides] a primer in the nucleic acid amplification reaction,

wherein the template-deficient oligonucleotide comprises one or more template-deficient nucleotides,

B1 wherein the number and composition of template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end of the template-deficient oligonucleotide is sufficient to allow the template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end alone to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction.

2. (Amended) The method of claim 1 wherein the one or more template-deficient nucleotides are at [or near] the 5' end of the template-deficient oligonucleotide.

19. (Amended) The method of claim 1 wherein the nucleic acid amplification reaction is selected from the group consisting of exponential rolling circle amplification (ERCA), [and] rolling circle amplification (RCA), multiple displacement amplification (MDA), strand displacement amplification (SDA), nucleic acid sequence based amplification (NASBA), B2 transcription-mediated amplification (TMA), polymerase chain reaction (PCR), self-sustained sequence replication (3SR), amplification with Qβ replicase, and cycle sequencing.

23. (Amended) A method of reducing formation of artifacts in a nucleic acid amplification reaction, the method comprising

B3 using a template-deficient oligonucleotide as [at least one of the oligonucleotides] a primer in the nucleic acid amplification reaction,

wherein the nucleic acid amplification reaction does not involve [cycle sequencing] thermal cycling.

27. (Amended) The method of [26] 23 wherein the nucleic acid amplification is rolling B4 circle amplification.

31. (Amended) The method of claim 23 wherein the nucleic acid amplification reaction is selected from the group consisting of exponential rolling circle amplification (ERCA), [and] rolling circle amplification (RCA), multiple displacement amplification (MDA), strand displacement amplification (SDA), nucleic acid sequence based amplification (NASBA), transcription-mediated amplification (TMA), [polymerase chain reaction (PCR),] self-sustained sequence replication (3SR), and amplification with Qβ replicase.

33. (Amended) The method of claim 32 wherein the one or more template-deficient nucleotides are at [or near] the 5' end of the template-deficient oligonucleotide.

[Please add the following new claims.]

77. (New) A method of reducing formation of artifacts in a nucleic acid amplification reaction, the method comprising

using a template-deficient oligonucleotide as a primer in the nucleic acid amplification reaction,

wherein the template-deficient oligonucleotide comprises one or more template-deficient nucleotides, wherein the two or more adjacent template-deficient nucleotides are within three nucleotides of the 5' end of the template-deficient oligonucleotide,

wherein the number and composition of template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end of the template-deficient oligonucleotide is sufficient to allow the template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end alone to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction.

78. (New) The method of claim 77 wherein the modified nucleotides are abasic nucleotides.

79. (New) A method of reducing formation of artifacts in a nucleic acid amplification reaction, the method comprising

using a template-deficient oligonucleotide as a primer in the nucleic acid amplification reaction,

wherein the template-deficient oligonucleotide comprises one or more template-deficient nucleotides, wherein the modified nucleotides are abasic nucleotides,

wherein the number and composition of template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end of the template-deficient oligonucleotide is sufficient to allow the template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end alone to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction.

80. (New) A method of reducing formation of artifacts in a nucleic acid amplification reaction, the method comprising

using a template-deficient oligonucleotide as a primer in the nucleic acid amplification reaction,

wherein the template-deficient oligonucleotide comprises one or more template-deficient nucleotides, wherein the two or more adjacent template-deficient nucleotides are within three nucleotides of the 5' end of the template-deficient oligonucleotide, wherein the modified nucleotides are abasic nucleotides,

wherein the number and composition of template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end of the template-deficient oligonucleotide is sufficient to allow the template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end alone to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction.

Please cancel claims 20, 24-26, 28-30 and 46-49.

Remarks

Claims 1-19, 21-23, 27, 31-45 and 50-80 are pending. Claims 1-19, 21-23, 27, 31-45 and 77-80 are under examination. Claims 20, 24-26, 28-30 and 46-49 have been canceled. Claims 50-76 have been withdrawn from consideration as being drawn to a non-elected invention. Claims 1, 2, 19, 23, 27, 31 and 33 have been amended. Claims 77-80 are newly added. Claims 1, 2, 19, 23, 27, 31 and 33 were amended to more clearly claim what applicants consider to be their invention. Claims 1 and 23 have been amended to recite use of a template-deficient oligonucleotide as a primer in a nucleic acid amplification reaction. This amendment finds support at least in original claim 20. Claims 2 and 33 were amended to recite that the one or more template-deficient nucleotides are at the 5' end of the template-deficient oligonucleotide. These amendments merely clarify the language of the claims. Claims 19 and 31 were amended to remove an extraneous "and" and thus correct a grammatical informality. Claim 23 was also amended to recite that the nucleic acid amplification reaction does not involve thermal cycling. This amendment finds support at least in original claim 26. Claim 27 was amended to depend from claim 23 rather than canceled claim 26. This amendment is merely a matter of form to maintain proper claim dependency. Claim 31 was also amended to eliminate reference to the polymerase chain reaction. This amendment merely makes claim 31 consistent with amended claim 23 (from which it depends).

Newly added claim 77 is an independent claim corresponding to the subject matter of original claims 1, 4 and 20. Newly added claim 78 is a dependent claim corresponding to the subject matter of original claims 1, 4, 9 and 20. Newly added claim 79 is an independent claim corresponding to the subject matter of original claims 1, 9 and 20. Newly added claim 80 is an independent claim corresponding to the subject matter of original claims 1, 4, 9 and 20.

A marked-up version of the amended claims is attached to this Amendment as a separate sheet as required by 37 C.F.R. § 1.121(c)(1)(ii). A copy of all of the pending claims as they are believed to have been amended is attached to this Amendment as an appendix. This copy of all

of the pending claims is provided only as a convenience and is not intended to be an amendment of the claims pursuant to 37 C.F.R. § 1.121(c)(3).

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 1-49 were rejected under 35 U.S.C. § 112, first paragraph, as not being enabled beyond the scope of the subject matter of claims 1, 4, 9 and 20. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

1. Applicants first note that, although the rejection indicates that the claims allegedly are enabled only for primers, there are no reasons provided to support this. It is not apparent why it would require undue experimentation for those of skill in the art to use oligonucleotides in the claimed method. Applicants submit that oligonucleotides having the features recited in the claims will be less prone to artifact generation in amplification reactions. Because some artifacts can be generated from oligonucleotides without priming, the claimed features can reduce artifact production without priming by the oligonucleotide. Notwithstanding this, applicants have amended the claims to recite explicitly the use of a template-deficient oligonucleotide as a primer in a nucleic acid amplification reaction.

2. Regarding the proximity of template-deficient nucleotides to the 5' end in the template-deficient oligonucleotides, the rejection alleges that, because there is no requirement that the 5' nucleotides could not also bind to a template, the 3' and 5' ends could bind to a non-target template and prime non-target synthesis, thus resulting in unwanted non-target amplification products. Applicants first note that it is not clear how this possibility relates to the 5' proximity of template-deficient nucleotides. Because template-deficient nucleotides can nevertheless be primer-capable (that is, capable of hybridizing to complementary nucleotides; see, for example, Figure 5 and compare the last paragraph on page 11 with the last full paragraph on page 12), the location of template-deficient nucleotides in the claimed template-deficient oligonucleotides need not affect the ability of the template-deficient oligonucleotides to bind to other nucleic acid strands. Rather, as the specification makes clear, template-deficient nucleotides prevent those nucleotide positions in the template-deficient oligonucleotides from serving as template nucleotides (which has the effect of stopping replication at that point). Thus, there is no basis for limiting the location of template-deficient nucleotides in the template-deficient oligonucleotides

based on any supposed distinction in hybridization ability of template-deficient oligonucleotides having template-deficient nucleotides closer to or further away from the 5' end.

Relevant to this, applicants next note that this aspect of the rejection is focused on a different problem than the problem addressed by the claimed method. In amplification reactions, a number of potential sources of undesired (e.g. non-target) nucleic acid replication have been observed and identified. Such "artifacts" of amplification reactions arise from a variety of mechanisms, some of which are general and other of which are specific to particular types of amplification. For example, it is known that primers may bind to (and prime from) both target sequences (having exact or nearly exact complementarity to primer sequences) and non-target sequences (having partial complementarity to primer sequences). A number of strategies are known to deal with various forms of artifact production. For example, it is known that adjusting the primer stability (length and composition) and matching this to reaction conditions (principally temperature) can favor exact (target-specific) hybridization over mismatch (non-target) hybridization. Significantly (in the case of the present rejection), those of skill in the art would know how to design primer length and composition to reduce the amount of non-target hybridization (and thus, reduce the amount of non-target priming). Similarly, those of skill in the art would know how to design primers to avoid hairpin formation unless a hairpin structure is desired in the primer, as is the case for some amplification techniques.

Applicants are not required to exclude from the claims that which those of skill in the art would know to avoid. See *In re Anderson*, 471 F.2d 1237, 1242 (CCPA 1973), ("[I]t is always possible to put something into a combination to render it inoperative. It is not a function of the claims to *exclude* all such matters but to point out what the combination is."); *Ex parte Cole*, 223 USPQ 94, 95-96 (PTO Bd. App. 1983) ("Claims are addressed to the person of average skill in the particular art. Compliance with 112 must be adjudged from that perspective, not in a vacuum. It is always possible to theorize some combination of circumstances which would render a claimed composition or method inoperative, but the art skilled would assuredly not choose such a combination."); *Horton v. Stevens*, 7 USPQ2d 1245, 1247 (Bd. Pat. App. & Int'f 1988) ("The mere fact that a claim embraces undisclosed or inoperative species or embodiments does not necessarily render it unduly broad."); and *Ex parte Breuer*, 1 USPQ2d 1906 at 1906

(Bd. Pat. App. & Int'f 1986) ("The issue is not whether the examiner can conjure up a substituent group...which does not exist. A person having ordinary skill in the art would readily appreciate that compounds containing such substituent group[s] do not exist....'[N]obody will use them...and the claims do not cover them'.").

The claims do not require the use of template-deficient oligonucleotides that would have non-functional characteristics such as significant non-target hybridization or significant hairpin formation. Those of skill in the art would know to avoid such designs when practicing the claimed method. No undue experimentation would be involved. This is all the law requires. Accordingly, applicants submit that the claims are fully enabled and are specifically enabled beyond the scope of claim 4.

3. Regarding the use of intercalating agents, applicants note that the claims only require (in some embodiments) that a template-deficient nucleotide be a nucleotide derivatized with an intercalating agent. That is, the template-deficient oligonucleotide used in the claimed method may include (as a template-deficient nucleotide) a nucleotide that has an intercalating agent attached (e.g. covalently coupled). The rejection appears to assume that the claim somehow requires that the intercalating agent is intercalated into the oligonucleotide. This is not the case. In particular, applicants note that an intercalating agent is a compound that is capable of intercalating into nucleic acids. Regardless of this capability, an intercalating agent remains a chemical compound and chemical compounds can be used to derivatize nucleotides independent of other functions or capabilities they may have. The fact that applicants chose to refer to a class of chemical compounds by a characteristic capability does not somehow incorporate that function into the claim as a means of synthesis of the template-deficient oligonucleotide. Because the claims do not require that the recited intercalating agents actually intercalate anywhere during the course of the method, there can be no issue of enablement based on this capability of the recited intercalating agents.

Regarding biotinylated nucleotides, applicants note that the claims require that the biotinylated nucleotide function as a template-deficient nucleotide. To the extent that there are some forms of biotinylated nucleotides that would be template-capable in the claimed method, the use of such forms of biotinylated nucleotides are not encompassed by the claims. Because

claims need only be enabled for what they encompass, there can be no enablement issue regarding biotinylated nucleotides that are not template-deficient when used in the claimed method. Accordingly, for all of the above reasons, applicants submit that the claims are fully enabled.

Rejection Under 35 U.S.C. § 112, second paragraph

Claims 2, 16 and 33 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

1. Claims 2 and 33 were considered indefinite in the recitation "wherein the one or more template-deficient nucleotides are at or near the 5' end" on the basis that the term "near" is indefinite. Applicants submit that those of skill in the art would understand what the recited phrase encompasses. Specifically, "near" means "in proximity to" and thus template-deficient nucleotides closer to the 5' end that to the 3' end would be understood to be "near" the 5' end. Notwithstanding this, applicants have amended claims 2 and 33 to eliminate reference to "near."

2. Claim 16 was considered indefinite in the recitation "the polymerase chain reaction" on the basis that the term lacks antecedent basis. Applicants note that 35 U.S.C. § 112, second paragraph, only requires that the claims be reasonably clear such that those of skill in the art can understand what is claimed. In the case of claim 16, applicants are referring to the well known amplification technique referred in the art as the polymerase chain reaction. There is no reasonable basis for asserting that those of skill in the art would interpret claim 16 in any other way nor that they would consider the phrase to be referring to an earlier, but unstated, element. Regarding the requirement that claim elements and terms that are later referred to must have antecedent basis, applicants note that no particular claim language is required to introduce a claim term.

Rejection Under 35 U.S.C. § 102

Claims 1, 2, 5, 11, 20, 21, 23, 28 32, 36, 39, 41-44, 46 and 49 were rejected under 35 U.S.C. § 102(a) as being anticipated by U.S. Patent No. 6,027,923 to Wallace et al. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The rejection asserts that Figure 2 of Wallace et al. shows that "effective priming is achieved with only that portion of the oligonucleotide that is 3' of the template-deficient nucleotide(s)", and that the paragraph bridging columns 4 and 5 of Wallace et al. discloses primers that contain elements that prevent the nucleic acid polymerase from replicating the entire sequence of the primer. Regarding Figure 2 of Wallace et al., applicants note that this figure does not depict or describe priming or primer binding. Rather, Figure 2 depicts the replication products formed by primer extension of the primer-bound nucleic acid strands depicted in Figure 1, step (d). Thus, contrary to the assertion in the rejection, Figure 2 of Wallace et al. does not support the present rejection.

Figures 1 through 4 of Wallace et al. show seven stages (steps (a) through (g)) of the replication of a target sequence using the method and primers disclosed by Wallace et al. In these figures, the original strands of the target sequence are depicted as solid lines, the primers are depicted by dashed lines, and replicated strands are depicted by dotted lines. Non-replicable nucleotides (i.e. template-deficient nucleotides) are depicted as an "x" or an "o." In Figures 1-4, the template-deficient nucleotides appear in the middle of the primers (see Figure 1, step (b), showing the primers hybridized to the two strands of the target sequence). As the primers are extended, the template-deficient nucleotides are incorporated into the replicated strands (see Figure 1, step (c) (dashed/dotted strands)). Because the primers are incorporated into the strands, the first replicated strands are "full length" (that is, they include all of the primer sequences and primer complement sequences). As a result, the primers are fully complementary to the first replicated strands as depicted in Figure 1, step (d), where primers are shown hybridized to the two original target sequence strands (top and bottom) and the two first replicated strands (middle two strands).

When the hybridized primers are extended on these four strands, the results differ for the original target sequence strands and the first replicated strands. As in the first round of replication, the original target sequence strands are fully replicated (see top and bottom double-stranded products in Figure 2, step (e)). However, the first replicated strands are only partially replicated (see middle two (partially) double-stranded products in Figure 2, step (e)). The partially replicated strands are marked with reference numbers 10 and 20. As can be seen,

replication terminated at the template-deficient nucleotides incorporated into the first replicated strands. Thus, two of the second replicated strands (reference numbers 10 and 20) are not full length and their ends do not have a full complement of the primer sequences.

Figure 3, step (f), depicts primer hybridization (**and the lack of primer hybridization**) to the eight strands from Figure 2, step (e). As can be seen, the primers hybridize to, from top to bottom, the first two strands, the fourth strand, and the last three strands. The primers do not hybridize to the third and fifth strands (and replication of those strands is, as a consequence, not primed by the primers; see Figure 4, step (g)). The third and the fifth strands are the two second replicated strands that are not full length (reference numbers 10 and 20 in Figure 2). The primers do not hybridize because these strands have insufficient sequences complementary to the primers. The strands do have some sequence complementary to the primers. Specifically (and relevantly), **they have only sequence complementary to those nucleotides in the primers that are 3' of the template-deficient nucleotide**. Thus, as depicted in Figure 3 of Wallace et al., the number and composition of template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end of the primers used in Wallace et al. is **not** sufficient to allow the template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction of Wallace et al. This is exactly the opposite of what the present claims require.

Note that the template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end of the primers of Wallace et al. are the only nucleotides in the primer that are complementary to the second replicated strands that are not full length (reference numbers 10 and 20 in Figure 2). For this reason, the method and primers of Wallace et al. as depicted in Figures 1 through 4 clearly indicate that the primers of Wallace et al. specifically lack a feature recited in the present claims. In fact, a main goal of Wallace et al. in the use of primers having template-deficient nucleotides is to **prevent** priming of second generation and later replicated strands by the original primers (see column 2, lines 49 - 53, where Wallace et al. states that "...the second generation primer extension products contain at least a portion of the nucleic acid sequence of interest and **cannot serve as templates for the synthesis of extension products of the primers which were extended to synthesize their templates**." (emphasis added)). This is

exactly the opposite of the goal of the claimed method where replication of the replicated strands can be primed by the primers. Because Wallace et al. does not disclose every feature of the claimed method, Wallace et al. cannot anticipate the claimed method.

Claims 1, 3, 5, 11, 13-16, 19, 20, 23-25, 28-32, 34, 36, 37, and 41-43 were rejected under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent No. 6,140,055 Todd et al. Applications respectfully traverse this rejection to the extent that it is applied to the claim as amended.

1. The rejection asserts that the independent claims "place no lower limit on the number of nucleotides that must be between the template-deficient nucleotides and the 3' terminus of the template-capable nucleotide[] segment." This is incorrect. The claims require that "the number and composition of template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end of the template-deficient oligonucleotide is sufficient to allow the template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end alone to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction." It is beyond question that this functional requirement places a lower limit on the number of nucleotides that must be between the template-deficient nucleotide(s) and the 3' end of the template-deficient oligonucleotide. Specifically, there must be enough template-capable nucleotides 3' of the template-deficient nucleotide(s) to make those template-capable nucleotides capable of effectively priming replication. It also cannot be questioned that there can be oligonucleotides with template-deficient nucleotides such that there are too few template-capable nucleotides 3' of the template-deficient nucleotides to effectively prime replication. For example, in an oligonucleotide having a single template-capable nucleotide 3' of template-deficient nucleotides, the single 3' template-capable nucleotide would clearly not be capable of effectively priming replication. Such an oligonucleotide is discussed in column 11 of Todd et al. (as discussed in the Amendment mailed September 4, 2001). Thus, the assertion that the claims place no lower limit on the number of nucleotides that must be between the template-deficient nucleotides and the 3' terminus of the oligonucleotide is incorrect and cannot support the present rejection.

2. The rejection refers to an example of a reporter substrate in Todd et al. (SEQ ID NO:4, column 9) and implies that this oligonucleotide is encompassed by the claims. Applicants first note this reporter substrate is not a primer or other extendible oligonucleotide as used in the

method of Todd et al. Rather, it is a substrate for cleavage as a way of detecting amplification of a target sequence. Significantly, the reporter substrate "has a 3' phosphate group which prevents its extension by DNA polymerase during PCR." Thus, the number and **composition** of 3' nucleotides in the reporter substrate of Todd et al. are **not** sufficient to effectively prime replication because the 3' end phosphate prevents extension. For at least this reason, the reporter substrate of Todd et al. does not have every feature of the claimed template-deficient oligonucleotides. Accordingly, for at least this reason, Todd et al. does not anticipate the present claims.

Applicants also note that the present claims are drawn to a method involving a nucleic acid amplification reaction and require use of a template-deficient oligonucleotide as a primer in the nucleic acid amplification reaction. Thus, at least one template-deficient oligonucleotide used in the claimed method must be a primer. The reporter substrate of Todd et al. cannot serve as a primer in a nucleic acid amplification reaction because it has a 3' phosphate that prevents extension. For at least this reason, the reporter substrate of Todd et al. does not have every feature of the claimed template-deficient oligonucleotides. Accordingly, for all of the above reasons, Todd et al. does not anticipate the present claims.

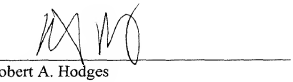
Pursuant to the above amendments and remarks, reconsideration and allowance of the pending application is believed to be warranted. The Examiner is invited and encouraged to directly contact the undersigned if such contact may enhance the efficient prosecution of this application to issue.

Payment in the amount of $284.00, with $200.00 representing the fee for a Request for Extension of Time for a small entity under 37 C.F.R. § 1.17(a)(2), and with $84.00 representing the fee for a excess independent claims for a small entity under 37 C.F.R. § 1.16(b), is to be charged to a credit card and such payment is authorized by the signed, enclosed document entitled Credit Card Payment Form PTO-2038. This amount is believed to be correct; however, the Commissioner is hereby authorized to charge any additional amount due or credit any overpayment to Deposit Account No. 14-0629.

Allowance of claims 1-19, 21-23, 27, 31-45 and 77-80 is respectfully solicited.

Respectfully submitted,

NEEDLE & ROSENBERG, P.C.



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I hereby certify that this correspondence, including any items indicated as attached or included, is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, Washington, D.C. 20231, on the date indicated below.

Robert A. Hodges

4/29/2002
Date

Appendix: Copy of All Pending Claims Under Examination After Amendment

1. (Amended) A method of reducing formation of artifacts in a nucleic acid amplification reaction, the method comprising

using a template-deficient oligonucleotide as [at least one of the oligonucleotides] a primer in the nucleic acid amplification reaction,

wherein the template-deficient oligonucleotide comprises one or more template-deficient nucleotides,

wherein the number and composition of template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end of the template-deficient oligonucleotide is sufficient to allow the template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end alone to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction.

2. (Amended) The method of claim 1 wherein the one or more template-deficient nucleotides are at [or near] the 5' end of the template-deficient oligonucleotide.

3. The method of claim 1 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are adjacent.

4. The method of claim 3 wherein the two or more adjacent template-deficient nucleotides are within three nucleotides of the 5' end of the template-deficient oligonucleotide.

5. (Amended) The method of claim 1 wherein the template-deficient nucleotides are selected from the group consisting of modified nucleotides, derivatized nucleotides, ribonucleotides, and nucleotide analogs.

6. (Amended) The method of claim 1 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are different.

7. The method of claim 1 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are template-deficient for different reasons.

8. The method of claim 5 wherein the template-deficient nucleotides are modified nucleotides.

9. The method of claim 5 wherein the modified nucleotides are abasic nucleotides.

10. The method of claim 5 wherein the template-deficient nucleotides are selected from the group consisting of abasic nucleotides, nucleotides with an inverted base, fluoro substituted nucleotides, alkyl substituted nucleotides, nucleotides with phenyl substituted ethers, nucleotides with substituted thioethers, nucleotides with phosphate esters, α-nucleotides, 2',3'-dideoxy nucleotides, ribonucleotides, nucleotides derivatized with biotin, nucleotides derivatized with amine, nucleotides derivatized with Hex, nucleotides derivatized with Tet, nucleotides derivatized with Fam, nucleotides derivatized with fluorescein, nucleotides derivatized with rhodamine, nucleotides derivatized with alkaline phosphatase, nucleotides derivatized with horseradish peroxidase, nucleotides derivatized with spacers, nucleotides derivatized with cholesteryl, nucleotides derivatized with DNP-TEG, nucleotides derivatized with psoralen cross-linkers, nucleotides derivatized with intercalating agents, and nucleotides derivatized with PNA conjugates.

11. The method of claim 1 wherein the nucleic acid amplification reaction does not involve cycle sequencing.

12. The method of claim 11 wherein the nucleic acid amplification reaction does not require linear amplification via thermal cycling.

13. The method of claim 12 wherein the nucleic acid amplification reaction does not involve linear amplification via thermal cycling.

14. The method of claim 1 wherein the nucleic acid amplification reaction involves exponential amplification via thermal cycling.

15. The method of claim 14 wherein the nucleic acid amplification reaction requires exponential amplification via thermal cycling.

16. The method of 14 wherein the nucleic acid amplification reaction involves the polymerase chain reaction.

17. The method of claim 1 wherein the nucleic acid amplification does not involve thermal cycling.

18. The method of 17 wherein the nucleic acid amplification is rolling circle amplification.

19. (Amended) The method of claim 1 wherein the nucleic acid amplification reaction is selected from the group consisting of exponential rolling circle amplification (ERCA), [and] rolling circle amplification (RCA), multiple displacement amplification (MDA), strand displacement amplification (SDA), nucleic acid sequence based amplification (NASBA), transcription-mediated amplification (TMA), polymerase chain reaction (PCR), self-sustained sequence replication (3SR), amplification with Qβ replicase, and cycle sequencing.

21. The method of claim 20 wherein all of the primers used in the nucleic acid amplification reaction are template-deficient.

22. The method of claim 1 wherein all of the oligonucleotides used in the nucleic acid amplification reaction are template-deficient.

23. (Amended) A method of reducing formation of artifacts in a nucleic acid amplification reaction, the method comprising

using a template-deficient oligonucleotide as [at least one of the oligonucleotides] a primer in the nucleic acid amplification reaction,

wherein the nucleic acid amplification reaction does not involve [cycle sequencing] thermal cycling.

27. (Amended) The method of [26] 23 wherein the nucleic acid amplification is rolling circle amplification.

31. (Amended) The method of claim 23 wherein the nucleic acid amplification reaction is selected from the group consisting of exponential rolling circle amplification (ERCA), [and] rolling circle amplification (RCA), multiple displacement amplification (MDA), strand displacement amplification (SDA), nucleic acid sequence based amplification (NASBA), transcription-mediated amplification (TMA), [polymerase chain reaction (PCR),] self-sustained sequence replication (3SR), and amplification with Qβ replicase.

32. The method of claim 23 wherein the template-deficient oligonucleotide comprises one or more template-deficient nucleotides.

33. (Amended) The method of claim 32 wherein the one or more template-deficient nucleotides are at [or near] the 5' end of the template-deficient oligonucleotide.

34. The method of claim 32 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are adjacent.

35. The method of claim 34 wherein the two or more adjacent template-deficient nucleotides are within three nucleotides of the 5' end of the template-deficient oligonucleotide.

36. The method oligonucleotide of claim 32 wherein the template-deficient nucleotides are selected from the group consisting of modified nucleotides, derivatized nucleotides, ribonucleotides, and nucleotide analogs.

37. The method oligonucleotide of claim 32 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are different.

38. The method of claim 32 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are template-deficient for different reasons.

39. The method of claim 36 wherein the template-deficient nucleotides are modified nucleotides.

40. The method of claim 36 wherein the modified nucleotides are abasic nucleotides.

41. The method of claim 36 wherein the template-deficient nucleotides are selected from the group consisting of abasic nucleotides, nucleotides with an inverted base, fluoro substituted nucleotides, alkyl substituted nucleotides, nucleotides with phenyl substituted ethers, nucleotides with substituted thioethers, nucleotides with phosphate esters, (α-nucleotides, 2',3'-dideoxy nucleotides, ribonucleotides, nucleotides derivatized with biotin, nucleotides derivatized with amine, nucleotides derivatized with Hex, nucleotides derivatized with Tet, nucleotides derivatized with Fam, nucleotides derivatized with fluorescein, nucleotides derivatized with rhodamine, nucleotides derivatized with alkaline phosphatase, nucleotides derivatized with horseradish peroxidase, nucleotides derivatized with spacers, nucleotides derivatized with cholesteryl, nucleotides derivatized with DNP-TEG, nucleotides derivatized with psoralen cross-linkers, nucleotides derivatized with intercalating agents, and nucleotides derivatized with PNA conjugates.

42. The method of claim 32 wherein the number and composition of template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end is sufficient to allow the template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end alone to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction.

43. The method of claim 23 wherein the template-deficient oligonucleotide is a primer.

44. The method of claim 43 wherein all of the primers used in the nucleic acid amplification reaction are template-deficient.

45. The method of claim 23 wherein all of the oligonucleotides used in the nucleic acid amplification reaction are template-deficient.

77. (New) A method of reducing formation of artifacts in a nucleic acid amplification reaction, the method comprising

using a template-deficient oligonucleotide as a primer in the nucleic acid amplification reaction,

wherein the template-deficient oligonucleotide comprises one or more template-deficient nucleotides, wherein the two or more adjacent template-deficient nucleotides are within three nucleotides of the 5' end of the template-deficient oligonucleotide,

wherein the number and composition of template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end of the template-deficient oligonucleotide is sufficient to allow the template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end alone to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction.

78. (New) The method of claim 77, wherein the modified nucleotides are abasic nucleotides.

79. (New) A method of reducing formation of artifacts in a nucleic acid amplification reaction, the method comprising

using a template-deficient oligonucleotide as a primer in the nucleic acid amplification reaction,

wherein the template-deficient oligonucleotide comprises one or more template-deficient nucleotides, wherein the modified nucleotides are abasic nucleotides,

wherein the number and composition of template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end of the template-deficient oligonucleotide is sufficient to allow the template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end alone to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction.

80. (New) A method of reducing formation of artifacts in a nucleic acid amplification reaction, the method comprising

using a template-deficient oligonucleotide as a primer in the nucleic acid amplification reaction,

wherein the template-deficient oligonucleotide comprises one or more template-deficient nucleotides, wherein the two or more adjacent template-deficient nucleotides are within three nucleotides of the 5' end of the template-deficient oligonucleotide, wherein the modified nucleotides are abasic nucleotides,

wherein the number and composition of template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end of the template-deficient oligonucleotide is sufficient to allow the template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end alone to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction.